Clinical Implications of Chromosomal Abnormalities in Gastric Adenocarcinomas

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Gastric carcinoma (GC) is one of the most common malignancies worldwide and has a very poor prognosis. Genetic imbalances in 62 primary gastric adenocarcinomas of various histopathologic types and pathologic stages and six gastric cancer–derived cell lines were analyzed by comparative genomic hybridization, and the relationship of genomic abnormalities to clinical features in primary GC was evaluated at a genome-wide level. Eighty-four percent of the tumors and all six cell lines showed DNA copy number changes. The recurrent chromosomal abnormalities including gains at 15 regions and losses at 8 regions were identified. Statistical analyses revealed that gains at 17q24-qter (53%), 20q13-qter (48%), 1p32–p36 (42%), 22q12-qter (27%), 17p13-pter (24%), 16p13-pter (21%), 6p21-pter (19%), 20p12-pter (19%), 7p21-pter (18%), 3q28-qter (8%), and 13q13–q14 (8%), and losses at 18q12-qter (11%), 3p12 (8%), 3p25-pter (8%), 5q14–q23 (8%), and 9p21-p23 (5%), are associated with unique patient or tumor-related features. GCs of differing histopathologic features were shown to be associated with distinct patterns of genetic alterations, supporting the notion that they evolve through distinct genetic pathways. Metastatic tumors were also associated with specific genetic changes. These regions may harbor candidate genes involved in the pathogenesis of this malignancy.

INTRODUCTION

Gastric cancer (GC) is highly prevalent, and represents the second most common cause of cancer-related death in the world (Whelan et al., 1993). At present, curative surgery remains the most effective treatment. Although radical lymph node dissection has been performed on patients with potentially curable carcinoma of the stomach to improve survival, prognosis remains poor because most patients have advanced disease at diagnosis (Wu et al., 1996a,b, 1997). Therefore, it is important to gain a further understanding of the biology of GC and the genetic alterations underlying this malignancy.

GC, like many other epithelial-derived cancers, results from the accumulation of multiple genetic alterations (Tahara, 1995). However, genetic changes involved in gastric carcinogenesis or progression have not yet been clearly defined. Increased DNA content and aneuploidy are frequently observed in GC cells (Brito et al., 1993). Numerical chromosomal anomalies described in GC include polysomy of chromosomes X, 1, 2, 3, 4, 15, 17, and 20, trisomy of chromosomes 9 and 11, and monosomy of chromosomes 7, 10, 17, and 18 (Han et al., 1996). Genetic instability resulting from defects in the mismatch repair genes plays an important role in GC progression (Lin et al., 1995; Dos Santos et al., 1996; Wu et al., 1998, 2000). Amplification and overexpression of oncogenes, such as *KSAM* (Nakatani et al., 1990), *ERBB2* (Mizutani et al., 1993; Uchino et al., 1993; Wu et al., 2000), and *MET* (Soman et al., 1991), as well as activation of *RASK* are frequent events (Kihana et al., 1991). Loss of heterozygosity (LOH) of loci corresponding to the *DCC* and *APC* genes is frequently detected (Tamura et al., 1993). The tumor suppressor gene *TP53* is mutated in about 40% of GCs (Kim et al., 1991; Labrecque et al., 1993; Renault et al., 1993). Amplification of the *CCNE* gene is also observed (Tahara, 1995). By use of the

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technique of comparative genomic hybridization (CGH), several investigators have reported deletions and amplifications at various chromosomal regions (Kobayashi et al., 1996; Kokkola et al., 1997), suggesting that additional unknown genetic alterations are involved in GC development.

Because CGH bypasses the need for tissue culture, it has been applied in the detection of recurrent chromosomal alterations in many solid tumor types, including GC (Knuutila et al., 1998a; Rooney et al., 1999). In GC, consistent DNA copy number gains are frequent on 20q, 17q, and 8q, whereas loss of 4q and 18q genetic material is common (Kokkola et al., 1997; El-Rifai et al., 1998; Sakakura et al., 1999). A comprehensive analysis of the relation between DNA copy number changes and clinical histopathologic features is lacking. Therefore, this study was designed to provide a detailed global view of genetic imbalances in a large panel of primary gastric adenocarcinomas (62 cases), to evaluate the clinical relevance of the identified chromosomal changes that may be implicated in the initiation, promotion, and progression of GC.

MATERIALS AND METHODS

Tumor Specimens and Patients

Tissue was obtained after surgical resection of 62 primary gastric carcinomas from the Veterans General Hospital–Taipei after signed informed consent. Table 1 shows the clinical and histopathologic data of the 62 cases. There were 47 men and 15 women, with a mean age of 65 years (range: 30–85). All specimens were snap-frozen immediately after resection and stored at −70°C until use. Part of the sample was allocated for DNA extraction. The remaining tissue was fixed in 10% buffered formalin for histologic examination. Hematoxylin–eosin-stained sections were used to categorize tumors according to the classifications of Lauren and Ming (Lauren, 1965; Ming, 1977). All cases were histologically confirmed as gastric adenocarcinomas, and the tumor specimens consisted predominantly of cancer.

DNA Extraction

Genomic DNA was isolated from tumor specimens by a standard proteinase K digestion and phenol/chloroform extraction procedure (Sambrook et al., 1989). Normal placenta DNA (Sigma, St. Louis, MO) was used as reference DNA.

Comparative Genomic Hybridization (CGH)

CGH was performed according to a reported protocol with some modifications (Kallioniemi et al., 1994). Briefly, target metaphase cells were obtained from phytohemagglutinin-stimulated peripheral blood lymphocytes from a healthy male donor and treated with proteinase K (0.1 μg/ml in 20 mM Tris–HCl, 2 mM $CaCl_2$, pH 7.5). Tumor DNA and reference placental DNA were labeled with FITC-12-dUTP and Spectrum Red-dUTP (Vysis, Downers Grove, IL), respectively, by nick translation. The final size of the labeled fragments was 300–3,000 bp. Equal amounts (200 ng) of tumor and reference DNA probes were mixed and precipitated with 10 μg of unlabeled Cot-1 DNA (Life Technologies, Gaithersburg, MD). DNA samples were dissolved in 10 μl of hybridization solution (70% formamide, 2.8× SSC, and 14.3% dextran sulfate, pH 7.0), and denatured for 7 min at 76°C. Metaphase slides were denatured in 70% formamide and 2× SSC (pH 7.0) for 3 min at 76°C and dehydrated through graded ethanol. Hybridization of DNA to the metaphase chromosomes was carried out for 2 days at 37°C in a moist chamber, and then washing twice in 50% formamide and 2× SSC (pH 7.0) at 43°C, followed by one wash in 2× SSC and three washes in 0.1 M phosphate buffer (pH 8.0) containing 0.1% NP40 at room temperature. Slides were counterstained with 4,6-diamidino-2-phenylindole (DAPI, 0.05 μg/ml) in Vectashield antifade solution (Vector Laboratories, Burlingame, CA).

For each hybridization, images of 6–10 metaphases were acquired with a computer-driven cooled CCD color camera (Photometrics, Tucson, AZ) attached to a microscope equipped with a standard fluorescence system (Axioscope; Zeiss, Jena, Germany), triple-band pass beam splitter, and emission filters (P-1 filter set; Chroma Technology, Brattleboro, VT). Fluorescence ratio profiles for each chromosome were calculated using the Quantitative Image Processing System (QUIPS; Vysis). Gains or losses of chromosomes were detected on the basis of the ratio profile deviating from the green to red balance value of 1.0. The upper and lower threshold limits for defining chromosomal gains and losses were set to 1.20 and 0.80, respectively. A ratio above 1.50 was termed amplification, and 95% confidence intervals were calculated. Metaphase green and red colors on both homologous chromosomes were selected for evaluation. Chromosomes X and Y were excluded from analysis, and chromosome 19 was also

TABLE 1. Clinical Data on 62 Cases of Gastric Adenocarcinoma*

Case no.	Sex/age	Tumor size (cm)	Site	Depth	Histology		Nuc. grade	INF	Lym. node meta.	Liver meta.	Vessel inv.	Lym. duct inv.	Peri. dissem.	TNM stage
					Lauren	Ming								
1	F/54	6.0	M	se	Diff	Inf	P	γ	+	−	−	+	−	IIIb
2	M/72	5.0	L	se	Diff	Inf	P	γ	−	−	−	+	−	II
3	M/71	5.0	L	se	Int	Inf	P	γ	+	−	−	+	−	IIIa
4	M/72	6.6	L	mp	Diff	Exp	P	β	−	−	−	−	−	Ib
5	F/42	4.5	M	se	Diff	Inf	P	γ	−	−	−	+	−	II
6	M/67	7.3	L	mp	Diff	Inf	P	γ	+	−	−	+	−	II
7	M/73	10.0	M	se	Int	Exp	P	β	+	+	−	−	−	IV
8	M/68	9.5	L	se	Diff	Exp	P	γ	+	−	−	+	−	II
9	M/73	11.6	M	sei	Diff	Exp	P	α	+	+	−	+	−	IV
10	F/71	7.4	M	mp	Int	Exp	M	α	−	−	−	+	−	Ib
11	M/67	7.0	M	se	Int	Exp	M	β	+	−	−	+	−	IIIa
12	M/71	11.0	M	se	Diff	Inf	P	γ	+	−	−	+	−	IIIa
13	M/75	9.3	M	se	Diff	Inf	P	γ	−	−	+	+	+	IV
14	F/57	5.6	U	se	Int	Inf	W	γ	+	−	−	+	−	IIIb
15	M/75	7.1	L	se	Int	Inf	M	γ	+	−	−	+	−	IIIb
16	M/85	7.5	L	se	Int	Exp	M	α	+	−	−	−	+	IV
17	M/72	6.0	U	se	Int	Inf	P	β	−	−	−	+	−	II
18	M/68	6.0	M	se	Diff	Inf	P	γ	−	−	−	+	−	II
19	M/69	5.0	L	se	Diff	Exp	P	β	+	−	−	+	−	IIIa
20	F/53	4.5	L	mp	Int	Exp	M	β	−	−	−	+	−	Ib
21	M/75	8.5	L	sei	Diff	Inf	P	γ	+	+	−	+	−	IV
22	M/60	6.0	L	se	Int	Inf	W	γ	−	−	−	+	−	IIIa
23	M/80	8.1	U	se	Int	Exp	W	β	−	−	−	+	−	IIIa
24	M/59	5.8	U	se	Int	Exp	P	α	−	−	−	+	−	IIIb
25	M/69	9.5	M	se	Diff	Exp	P	β	−	−	−	+	−	IIIb
26	F/71	11.0	L	si	Diff	Inf	P	γ	−	−	−	+	−	IV
27	M/61	9.0	L	se	Diff	Inf	P	γ	+	−	−	+	−	IIIb
28	F/77	8.0	L	se	Diff	Exp	P	α	−	−	+	+	−	II
29	M/66	5.3	U	se	Int	Inf	M	β	+	−	−	+	−	IIIb
30	M/66	8.0	U	se	Int	Inf	P	γ	+	−	−	+	+	IV
31	M/71	3.5	U	se	Int	Inf	M	α	+	−	+	+	−	IIIb
32	M/61	7.0	L	se	Int	Exp	P	β	+	−	−	+	−	IIIb
33	M/55	7.0	U	se	Int	Inf	P	γ	−	−	−	+	−	II
34	M/73	6.0	L	se	Int	Inf	M	γ	+	−	−	+	−	IIIb
35	M/76	8.6	L	se	Int	Inf	P	γ	+	−	−	+	−	IIIb
36	M/68	6.0	L	se	Diff	Inf	P	γ	+	−	−	+	−	IIIa
37	M/73	6.6	M	se	Int	Inf	M	γ	+	−	−	+	−	IIIb
38	F/63	7.6	L	se	Diff	Inf	P	γ	+	−	−	+	−	IIIa
39	M/56	4.4	L	se	Int	Inf	P	γ	+	−	−	+	−	IIIa
40	F/57	5.0	L	sei	Diff	Inf	P	γ	+	−	+	+	−	IV
41	M/67	9.5	M	se	Int	Inf	M	β	+	−	−	+	−	IIIa
42	F/69	6.0	U	se	Diff	Inf	P	γ	+	−	−	+	+	IV
43	F/63	5.0	L	se	Diff	Inf	P	γ	+	−	−	+	−	IIIb
44	M/82	7.0	L	si	Int	Inf	P	γ	+	+	−	+	+	IV
45	M/76	2.7	M	se	Int	Inf	M	γ	−	−	−	+	−	II
46	M/73	5.8	L	se	Diff	Inf	P	γ	+	−	−	+	−	IIIa
47	F/69	8.4	L	mp	Int	Exp	M	α	−	−	−	−	−	Ib
48	M/47	10.1	L	se	Diff	Inf	P	γ	+	−	−	+	−	IIIa
49	M/46	10.3	M	si	Diff	Inf	P	γ	+	−	−	+	+	IV
50	M/44	8.4	M	se	Int	Inf	P	γ	+	−	−	+	+	IIIa
51	M/54	7.5	L	sei	Int	Inf	P	γ	+	−	−	+	−	IV
52	F/57	5.6	L	sei	Diff	Inf	P	γ	+	−	−	+	+	IV
53	M/72	7.9	L	si	Int	Inf	M	β	+	−	−	+	+	IIIb
54	M/75	5.9	L	se	Int	Exp	M	β	+	−	−	+	−	IIIa
55	F/39	9.5	M	se	Diff	Exp	P	γ	+	−	−	+	+	IV
56	M/71	7.7	L	se	Diff	Inf	P	γ	+	−	−	+	−	IIIa
57	M/71	3.5	L	sm	Int	Exp	M	β	−	−	−	−	−	Ia
58	M/70	7.7	L	mp	Int	Exp	M	β	+	−	−	+	−	II
59	M/30	6.0	L	se	Diff	Exp	P	β	−	−	−	+	−	Ib
60	M/67	7.0	L	se	Int	Inf	P	γ	−	−	−	+	−	II
61	F/52	6.7	L	se	Int	Exp	M	β	−	−	−	+	−	II
62	M/67	5.6	M	se	Int	Inf	P	β	+	−	−	+	−	II

*F, female; M, male; U, upper third; M, middle third; L, lower third; sm, submucosa; mp, proper muscle; se, serosa exposure; sei, serosa exposure and infiltration; si, serosa infiltration; Diff, diffuse; Int, intestinal; Exp, expanding; Inf, infiltrating; P, poorly differentiated; M, moderately differentiated; W, well-differentiated; INF, pattern of tumor infiltration into the surrounding tissue: INF α (Infiltration Alpha), the tumor showing expanding growth and a distinct border with the surrounding tissue; INF β (Infiltration Beta), the tumor categorized between Infiltration Alpha and Infiltration Gamma; INF γ (Infiltration Gamma), the tumor showing infiltrating growth and an indistinct border from the surrounding tissue; Lymph node metastasis: −, negative; +, positive; Liver metastasis: −, negative; +, positive; Vessel invasion: −, negative; +, positive; Lymphatic duct invasion: −, negative; +, positive; Peritoneal dissemination: −, negative; +, positive; AJCC/UICC (American Joint Committee on Cancer/International Union Against Cancer) stage.

TABLE 2. DNA Copy Number Changes in 62 Cases of Gastric Adenocarcinoma and 6 Gastric Cancer-Derived Cell Lines

Cell lines/ primary tumors	Gains	Losses
	Cell lines	
NUGC-3	1q21–q22, 1q31-qter, 6p21, 7p, 7cen–q21, 8q, 9q33-qter, 11p15, 11q12–q13, 12q23-qter, 20	3cen–p13, 3q13.1, 4p14, 4q13-qter, 5q14–q32, 10q21, 18q21-qter
SCM1	1p33-pter, 1q21–q22, 5p, 9q31-qter, 11p15, 11q12–q13, 12q23, 15q24-qter, 16p12-pter, 16q13–q22, 17p11.2-pter, 17q24-qter, 18p, 20q, 21q22, 22q13.2-qter	2p, 2q, 3q24, 4q, 6cen–q21, 8p, 8q13–q22, 9p13–p22, 10p11.2–p14, 13q, 18q12–q21
KatoIII	1p34, 3q21, 3q27–q29, 6p23-pter, 7pter–q21, 8q24, 9q22.3-qter, 10q25, 11q13-qter, 12p13, 17q11.2-qter, 20	2cen–q22, 3p13, 4p15-qter, 5p14-pter, 5q14–q23, 10q21.2, 11p12–p15.3, 18q
AGS	1q, 20q	18q12–q22
AZ521	1p31–p36.1, 1q21, 17q12-qter	—
HR	1p34-pter, 11q12–q13, 17p13, 17p11.2-qter, 20p13	4q24–q32, 9p21–p22
	Primary tumors	
1	1p34-pter, 11q12–q13, 14q32, 15q24-qter, 17q24-qter, 20q13, 21q22	—
2	—	—
3	1p34-pter, 7p13, 7q21–q22, 11q12–q13	—
4	1q, 6p21-pter, 6q22–q24, 9p22-pter, 9q12-qter, 18p11.3, 20p12-pter, 20q12-qter	—
5	1q12–q21, 3q28-qter, 20p13	—
6	6p21, 7cen–q11, 8q11–q12	—
7	6p21-pter, 13q13–q14, 17q12–21, 20p, 20q	—
8	—	—
9	6p23–p25, 6cen–p21, 7pter–q11, 20p, 20q	5q14-qter, 9p21-pter, 9q21–q22, 18q12-qter
10	—	—
11	1p36, 16p	18q12-qter
12	1cen–q25, 1q32-qter, 7p21-pter, 17q11.2–q21, 17q24–q25, 20q13	—
13	1q12–q21, 17q11.2–q21, 17q24–q25	—
14	17cen-qter	18q12-qter
15	17q11.2–q21, 17q24–q25	—
16	6p22-pter, 7p12-pter, 10q25-qter, 11p15-pter, 18p11.3	—
17	1q22–q31, 2q24-qter, 6q22–q27, 8q13-qter, 11q13-qter, 17q12–q21, 20q12-qter	—
18	11q13, 17q11.2–q21, 17q24–q25	—
19	5p13, 6p23-pter, 7p14-pter, 7cen–q22, 8q21–q24, 9p22-pter, 10q25–q26, 17pter–q21.3, 17q24–q25, 20p, 20q	—
20	17q12-qter	—
21	1q12–q21, 11q12–q13, 17cen–q24, 18p11.2-pter, 20q3p25–p26	—
22	17cen–q21, 20q	—
23	1p32-pter, 1q12–q23, 1q32-qter, 2q37, 6p21, 7p21–p22, 7q11, 8q23–q24, 9q34, 11q12–q13, 13q12–q14, 13q34, 16p11.2-pter, 17p13, 17q, 20q	4p12–p15, 4q, 5q14–q23, 18q12-qter
24	9q	18q21-qter
25	1p32-pter, 9q34, 16p13, 17p13, 17q11.2–q21, 17q24–q25, 20q13, 22q12-qter	—
26	1p32-pter, 3q28–q29, 6p21, 8q24, 9q34, 11p15, 11q12–q13, 16p13, 16q23-qter, 17pter–q21, 17q23-qter, 22q	3p25–p26, 4q13–q32, 5q21–q23, 13q21–q32
27	1p32-pter, 1q, 2q37, 7p21–p22, 9q34, 11p15, 11q12–q13, 15q22–q24, 16p12-pter, 17pter–q21, 17q24–q25, 22q	3p12
28	1p34-pter, 6p21, 7p22, 7q11, 9q34, 17pter–q21, 17q23-qter, 20p, 20q, 22q	4p14-p15, 4q13-qter, 13q21–q22
29	1p34-pter, 1q21–q23, 1q41-qter, 6p21, 7p21–p22, 8p21–p22, 10p13-pter, 10q25, 16p, 16q23-qter, 17q, 20p11-qter, 22q12-qter	—
30	1p32-pter, 2q37, 6p21, 9q34, 11q12–q13, 12q24, 15q22–q24, 16p, 17p, 17q, 20p, 20q, 22q	3cen–p12, 5q14–q23, 9p21–p23, 13q21–q31
31	1p32-pter, 1q21–q23, 9q34, 16p13, 17p, 17q, 20q12-qter, 22q12-qter	13q21–q31
32	9q34, 11p13–q13, 13q12–q14, 17q12–q21, 20q	—

(Continued)

TABLE 2. DNA Copy Number Changes in 62 Cases of Gastric Adenocarcinoma and 6 Gastric Cancer-Derived Cell Lines (Continued)

Cell lines/ primary tumors	Gains	Losses
33	1p32-pter, 9q34, 16p, 16q23-qter, 17p, 17q, 20q12-qter, 22q12-qter	3p12, 6q12–q16, 13q21–q22
34	1p32-pter, 8q24, 9q34, 11q12–q13, 16p, 16q23-qter, 17p, 17q, 20q, 22q	3p26, 4p14–p15, 4q13–q32, 6q12–q16, 13q21–q31
35	1p35-pter, 9q34, 11q12–q13, 16p13, 17q24–q25, 20cen–q12, 22q12-qter	—
36	1p32-pter, 17q21-qter	—
37	1p32-pter, 1cen–q23, 1q32–q41, 3p21, 8p12–p22, 8q24, 9q34, 10p11–p12, 10q21–22, 10q25-qter, 11q12–q13, 17pter-q21, 17q24-qter, 20q	2q22–q32, 3p25–26, 4p13–p15, 4q13–q34, 12q21, 13q21–q22
38	1p32-pter, 1q21–q23, 8p21-pter, 9q34, 11q12–q13, 12p13, 16p, 17pter–q21, 17q24–q25, 20q12-qter, 22q12-qter	—
39	1p32–p35, 7p13-pter, 9q34, 13q13–q14, 17q24–q25, 20q13	—
40	1p32-pter, 16p13, 17p13, 17q12–q21, 17q24–q25, 22q13	—
41	1p34-pter, 17q11–q21, 17q24–q25, 20q11–12, 22q13	—
42	—	—
43	—	—
44	1p32-pter, 8q, 10p, 11q13–22, 13q32-qter	3p, 4p13–p15, 4q13–q32, 5q13–q23, 18q21-qter
45	—	—
46	1p32-pter, 9q34, 11q12–q13, 17pter–q21, 17q24-qter, 22q	13q21–q31
47	7p13-pter, 8q24, 20p12–p13	—
48	3q28–q29, 8q23-qter	—
49	1p32–p35, 1q12–q21, 3q27-qter, 6p21-pter, 7q22, 8q23-qter, 9cen–q21, 10pter–q22, 11q12–q13, 17q, 22q	3cen–p13, 4p15-qter, 9p13–p23, 13q21–q31, 14q12–q21
50	1p34-pter, 7p22, 9q34, 11q12–q13, 17p13, 17cen–q21, 17q24-qter, 20q13, 22q12–q13	—
51	1p34-pter, 11q12–q13, 17q12-qter, 20q12-qter	—
52	—	—
53	1q21-qter, 8p21–p22, 8q22-qter, 20p12-pter, 20q12-qter	—
54	1q21–q25, 12q13–q15, 17q12–q21, 17q24-qter, 20q	—
55	—	—
56	—	—
57	17q11–q21, 17q24-qter, 20q12-qter	18q22-qter
58	1q21–q23, 11q12–q13, 17q12–q21, 20q	13q21–q22
59	1q21–q31, 3q13-qter, 7p12–p21, 11p12-pter, 11q12–q23, 13q, 20p12-pter	—
60	—	—
61	1q21–q41	—
62	1p32-pter, 9q34, 11q12–q13, 16q23-qter, 17q12–q21, 17q24-qter, 20p12-pter, 20q, 22q13	—

excluded because of problematic interpretation of CG-rich areas on this chromosome.

Fluorescence In Situ Hybridization (FISH)

The yeast artificial chromosome (YAC) 934_E_1 clone containing DNA sequences of the human *MYC* gene was labeled with FITC-12-dUTP by nick translation and used as a probe for FISH analysis. The labeled probe (100 μg) was mixed with Cot-1 DNA in 10 μl of hybridization solution and hybridized to the metaphase slides as described above. Slides containing interphase nuclei from tumor or placenta were treated with RNase A (100 μg/ml) at 37°C for 1 hr before hybridization, after which the slides were washed, counterstained, and examined.

Statistical Analysis

The chi-square test was performed to test differences in the frequency of individual chromosomal changes in the tumor subgroups (Fisher's exact test was used when sample sizes were small). Factors considered include: age, sex, tumor size, tumor location, gross appearance (superficial, localized, or

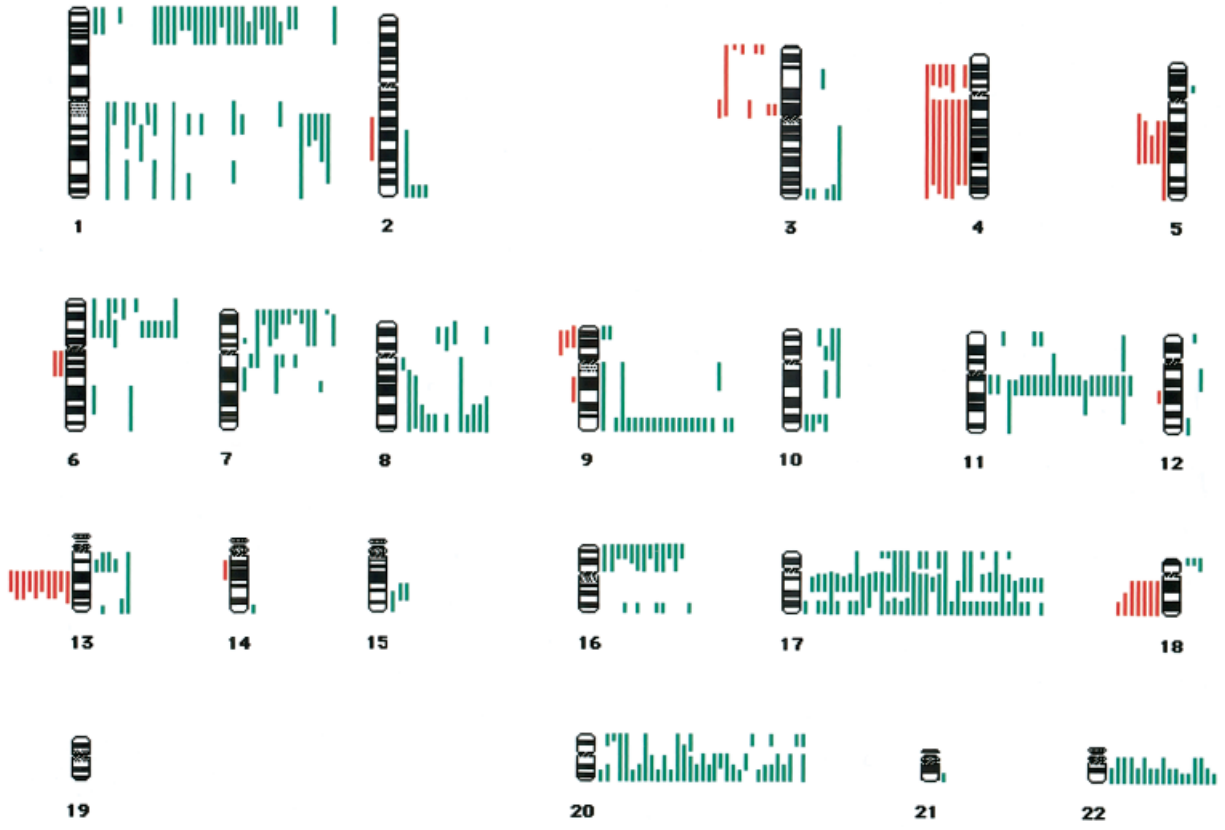

Figure 1. Summary of DNA copy number changes detected in 62 primary gastric adenocarcinomas by CGH. Vertical lines to the right of the chromosomes represent gains of genetic material; vertical lines to the left correspond to losses.

infiltrative), nuclear grade, mode and depth of cancer invasion, histologic classification according to Lauren and Ming, stromal reaction pattern, pattern of infiltration into surrounding tissue, lymphatic duct invasion, vascular invasion, lymph node metastasis, liver metastasis, and peritoneal dissemination. To examine independent chromosomal abnormalities associated with specific clinicopathologic features, multivariate analysis was performed based on the Cox proportional hazard model. All *P* values were two-tailed. Statistical significance was specified as $P < 0.05$.

RESULTS

Comparative Genomic Hybridization

A total of 62 cases of primary gastric adenocarcinomas and 6 gastric cancer-derived cell lines (AGS, AZ-521, HR, Kato-III, NUGC-3, and SCM-1) were analyzed by CGH. Abnormalities were found in 52 of the 62 (84%) tumors and in all cell lines (Table 2), with many abnormalities common to both. In primary tumors, the chromosome regions and subregions showing DNA copy number changes for the entire genome in the 52 informative cases are summarized in Figure 1. Recurrent gains were detected, in decreasing order of frequency, on 17q12–q21 (57% of the samples, 35/62), 17q24-qter (53%, 33/62), 20q13-qter (48%, 30/62), 1p32–p36 (42%, 26/62), 11q12–q13 (35%, 22/62), 9q34 (31%, 19/62), 22q12-qter (27%, 17/62), 17p13-pter (24%, 15/62), 16p13-pter (21%, 13/62), 7p21-pter (21%, 13/62), 6p21-pter (19%, 12/62), 20p12-pter (19%, 12/62), 8q23–q24 (18%, 11/62), 3q28-qter (8%, 5/62), and 13q13–q14 (8%, 5/62). Losses were detected on 13q21–q22 (16%, 10/62), 4q13–q32 (11%, 7/62), 18q12-qter (11%, 7/62), 4p14–p15 (10%, 6/62), 3p12 (8%, 5/62), 3p25-pter (8%), 5q14–q23 (8%, 5/62), and 9p21–p23 (5%, 3/62). High-copy-number amplifications (amplicons) were detected in five tumors, mapping to 8q23–q24 (2 cases), 9q34 (1 case), 11q12–q13 (1 case), and 19cen–q13.1 (1 case).

Interphase FISH

To confirm the CGH results, FISH using a locus-specific probe mapped to 8q24 was performed

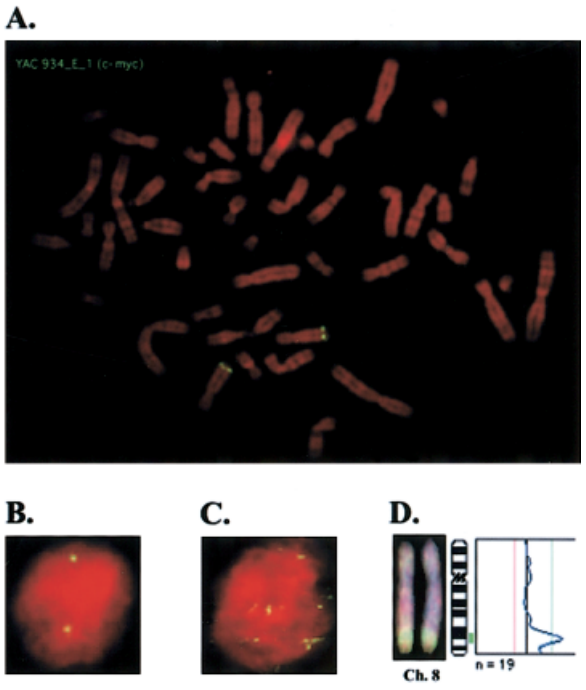

Figure 2. Detection of high copy-number amplifications of 8q by CGH and interphase FISH. FISH was performed using the YAC clone 934_E_1, which contains human DNA sequences of the *MYC* gene (A–C). **A:** Hybridization of the YAC probe to normal metaphase chromosomes at 8q24. **B:** Hybridization of the YAC probe to interphase placental cell. Representative examples of interphase FISH and fluorescent ratio image of CGH on GC case 49 are shown in **C** and **D**, respectively.

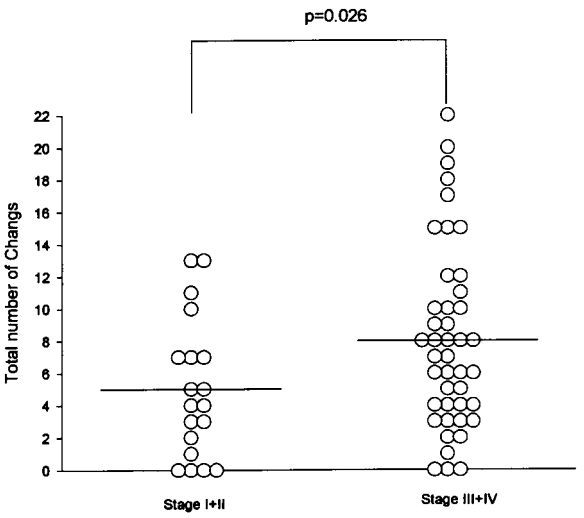

Figure 3. Distribution of the total number of chromosomal abnormalities for stage I/II and stage III/IV tumors. The total number of chromosomal arms involved in DNA copy number changes was calculated for each tumor, and comparison was made between early tumors (stages I–II) and advanced tumors (stages III–IV).

in cases showing gain at 8q. Representative results are shown in Figure 2. Interphase FISH analyses detected two alleles of the *MYC* gene in interphase placenta using the 8q24 locus-specific probe, whereas multiple signals were observed in the nuclei of tumor cells, suggesting that the increase in DNA copy numbers detected by CGH analysis represents true amplification events.

Association of Chromosome Copy Number Changes With Clinical Features

In the primary GC, the median number of chromosomes involved was 7.2 autosome arms per tumor (range 0–21). Chromosomal gains (in 84% of GC) were more commonly observed than were losses (in 31% of GC), with the mean value in gains of 5.4 autosome arms per tumor (range 0–16) and losses of 0.84 (range 0–6). The average number of chromosome arms involved was significantly higher in Stage III/IV tumors than in Stage I/II tumors (8.0 vs. 5.0, $P = 0.026$; Fig. 3).

To investigate whether DNA copy number changes at specific chromosomal regions may predominate in a specific pathway for gastric tumor initiation and/or progression, the relationship between recurrent chromosomal abnormalities and clinical features was evaluated. The frequency of chromosomal abnormalities involving gains on 1p, 3q, 6p, 7p, 8q, 9q, 11q, 13q, 16p, 17p, 17q, 20p, 20q, and 22q, and losses from regions on 3p, 4p, 4q, 5q, 9p, 13q, and 18q, were compared among different subtypes of GC according to their clinicopathologic data. In addition, gains in two subregions on 17q (17q12–q21 and 17q24-qter) and losses from two subregions on 3p (3p12 and 3p25-pter) were individually considered. Chromosomal abnormalities showing a significant association with specific subgroups of GC ($P < 0.05$) are summarized in Table 3. Univariate analysis showed that GC with differing histopathologic characteristics are associated with distinct patterns of genetic alterations, and chromosomal gain on 3q manifested as diffuse type GC ($P = 0.015$), whereas gains on 20q more frequently occurred in the intestinal type of GC by Lauren's classification ($P = 0.020$). Gains on 1p, 17q24-qter, and 22q manifested as infiltrating-type GC ($P = 0.014$, 0.025, and 0.034), whereas gains on 6p, 13q, and 20p, and loss of 18q12-qter, were more frequent in the expanding-type GC by Ming's criteria ($P = 0.046$, 0.041, 0.046, and 0.039).

GC showing different infiltrating growth patterns were also shown to be associated with distinct genetic changes. Gains on 7p and 20p were associated with tumors showing expansile growth into the surrounding tissues marked by a distinct border ($P = 0.034$ and 0.003), whereas tumors showing

TABLE 3. Relationship Between Clinical Data and Chromosome DNA Copy Number Changes

	+1p32–p36			+3q28-qter			+6p21-pter			+7p13-pter			+13q13–q14			+16p13-pter			+17p13-pter			+17q24-qter		
Factor[a]	−	+	*P*-value	−	+	*P*-value	−	+	*P*-value	−	+	*P*-value	−	+	*P*-value	−	+	*P*-value	−	+	*P*-value	−	+	*P*-value
Age																								
<65 years	12	9	0.916	17	4	**0.041***	20	1	**0.046***	17	4	1.000	18	3	0.325	17	4	1.000	16	5	1.000	10	11	1.000
≥65 years	24	17		40	1		30	11		32	9		39	2		32	9		31	10		19	22	
Site																								
upper	4	5	0.504	9	0	0.575	6	3	0.516	7	2	0.925	8	1	0.893	4	5	**0.021***	5	4	0.289	3	6	0.500
middle	9	8		15	2		14	3		14	3		16	1		15	2		14	3		7	10	
low	23	13		33	3		30	6		28	8		33	3		30	6		28	8		19	17	
Nuclear grade																								
w	2	1	0.746	3	0	0.274	2	1	0.572	2	1	0.821	2	1	0.123	2	1	0.803	2	1	0.732	1	2	0.891
m	11	6		17	0		15	2		14	3		17	0		13	4		14	3		8	9	
p	23	19		37	5		33	9		33	9		38	4		34	8		31	11		20	22	
Inf																								
α	5	2	0.418	7	0	0.565	4	3	0.081	3	4	**0.034***	7	0	**0.032***	6	1	0.898	5	2	0.673	5	2	0.307
β	12	6		17	1		13	5		14	4		14	4		14	4		15	3		9	9	
γ	19	18		33	4		33	4		32	5		36	1		29	8		27	10		15	22	
Ming classification																								
Expanding	17	4	**0.014***	20	1	0.654	14	7	**0.046***	14	7	0.087	17	4	**0.041***	18	3	0.514	17	4	0.551	14	7	**0.025***
Infiltrating	19	22		37	4		36	5		35	6		40	1		31	10		30	11		15	26	
Lauren classification																								
Intestinal	18	16	0.368	34	0	**0.015***	29	5	0.307	27	7	0.936	30	4	0.366	26	8	0.585	27	7	0.465	16	18	1.000
Diffuse	18	10		23	5		21	7		22	6		27	1		23	5		20	8		13	15	
Lymph node metastasis																								
Negative	16	5	**0.038***	18	3	0.325	17	4	1.000	17	4	1.000	19	2	1.000	17	4	1.000	16	5	0.96	12	9	0.242
Positive	20	21		39	2		33	8		32	9		38	3		32	9		31	10		17	24	
Lymphatic duct invasion																								
Negative	5	0	0.068	5	0	1.000	2	3	**0.046***	3	2	0.28	4	1	0.353	5	0	0.574	5	0	0.188	4	1	0.176
Positive	31	26		52	5		48	9		46	11		53	4		44	13		42	15		25	32	
Liver metastasis																								
Negative	33	25	0.633	53	5	1.000	48	10	0.166	46	12	1.000	54	4	0.292	45	13	0.571	43	15	0.564	26	32	0.332
Positive	3	1		4	0		2	2		3	1		3	1		4	0		4	0		3	1	
Vessel invasion																								
Negative	35	23	0.300	53	5	1.000	47	11	1.000	46	12	1.000	53	5	1.000	47	11	0.191	46	12	**0.041***	29	29	0.116
Positive	1	3		4	0		3	1		3	1		4	0		2	2		1	3		0	4	
Peritoneal carcinoma																								
Negative	30	22	1.000	48	4	1.000	42	10	1.000	40	12	0.673	47	5	0.582	40	12	0.673	39	13	1.000	24	28	1.000
Positive	6	4		9	1		8	2		9	1		10	0		9	1		8	2		5	5	
Depth																								
sm	1	0	0.112	1	0	0.363	1	0	0.454	1	0	0.777	1	0	0.595	1	0	0.551	1	0	0.446	0	1	0.229
mp	6	0		6	0		4	2		5	1		6	0		6	0		6	0		5	1	
se	25	21		43	3		39	7		35	11		41	5		35	11		33	13		20	26	
si	4	5		7	2		6	3		8	1		9	0		7	2		7	2		4	5	
Stage																								
I	6	0	**0.023***	5	1	0.539	5	1	0.085	4	2	0.379	5	1	0.581	6	0	0.191	6	0	0.241	4	2	0.088
II	10	3		12	1		11	2		12	1		13	0		12	1		11	2		9	4	
III	12	17		28	1		26	3		21	8		26	3		20	9		19	10		9	20	
IV	8	6		12	2		8	6		12	2		13	1		11	3		11	3		7	7	

[a]See Table 1 for details.
*$P < 0.05$ (in boldface).

TABLE 3. Relationship Between Clinical Data and Chromosome DNA Copy Number Changes (Continued)

	+20p			+20q			+22q12-qter			−3p12			−3p25-pter			−5q14-q23			−9p21-p23			−18q12-qter		
Factor[a]	−	+	*P*-value	−	+	*P*-value	−	+	*P*-value	−	+	*P*-value	−	+	*P*-value	−	+	*P*-value	−	+	*P*-value	−	+	*P*-value
Age																								
<65 years	19	2	0.175	13	8	0.246	15	6	0.844	18	3	0.325	21	0	0.157	21	0	0.157	20	1	1.000	19	2	1.000
≥ 65 years	31	10		19	22		30	11		39	2		36	5		36	5		39	2		36	5	
Site																								
upper	7	2	0.817	3	6	0.369	5	4	0.400	7	2	0.241	9	0	0.509	7	2	0.241	8	1	0.112	6	3	0.062
middle	13	4		8	9		12	5		16	1		16	1		16	1		15	2		15	2	
low	30	6		21	15		28	8		34	2		32	4		34	2		36	0		34	2	
Nuclear grade																								
w	3	0	0.648	1	2	0.701	3	0	0.467	3	0	0.274	3	0	0.732	2	1	0.123	3	0	0.472	1	2	**0.007***
m	14	3		8	9		13	4		17	0		15	2		17	0		17	0		15	2	
p	33	9		23	19		29	13		37	5		39	3		38	4		39	3		39	3	
Inf																								
α	4	3	**0.003***	4	3	**0.012***	5	2	0.840	7	0	0.159	7	0	0.159	6	1	0.772	6	1	0.317	5	2	0.143
β	11	7		4	14		14	4		18	0		18	0		17	1		18	0		15	3	
γ	35	2		24	13		26	11		32	5		32	5		34	3		35	2		35	2	
Ming classification																								
Expanding	14	7	**0.046***	10	11	0.652	19	2	**0.034***	21	0	0.157	21	0	0.157	19	2	1.000	20	1	1.000	16	5	**0.039***
Infiltrating	36	5		22	19		26	15		36	5		36	5		38	3		39	2		39	2	
Lauren classification																								
Intestinal	28	6	0.708	13	21	**0.020***	25	9	0.854	31	3	1.000	31	3	1.000	31	3	1.000	33	1	0.585	28	6	0.116
Diffuse	22	6		19	9		20	8		26	2		26	2		26	2		26	2		27	1	
Lymph node metastasis																								
Negative	16	5	0.525	13	8	0.246	17	4	0.375	20	1	0.654	20	1	0.654	19	2	1.000	21	0	0.545	18	3	0.680
Positive	34	7		19	22		28	13		37	4		37	4		38	3		38	3		37	4	
Lymphatic duct invasion																								
Negative	2	3	**0.046***	2	3	0.667	5	0	0.310	5	0	1.000	5	0	1.000	5	0	1.000	5	0	1.000	4	1	0.462
Positive	48	9		30	27		40	17		52	5		52	5		52	5		54	3		51	6	
Liver metastasis																								
Negative	48	10	0.166	31	27	0.346	41	17	0.568	54	4	0.292	55	3	**0.030***	55	3	**0.030***	56	2	0.184	53	5	0.059
Positive	2	2		1	3		4	0		3	1		2	2		2	2		3	1		2	2	
Vessel invasion																								
Negative	47	11	1.000	30	28	1.000	44	14	0.059	53	5	1.000	53	5	1.000	53	5	1.000	55	3	1.000	51	7	1.000
Positive	3	1		2	2		1	3		4	0		4	0		4	0		4	0		4	0	
Peritoneal carcinoma																								
Negative	42	10	1.000	26	26	0.733	38	14	1.000	50	2	**0.026***	49	3	0.180	49	3	0.180	51	1	0.065	46	6	1.000
Positive	8	2		6	4		7	3		7	3		8	2		8	2		8	2		9	1	
Depth																								
sm	1	0	0.765	0	1	0.634	1	0	0.390	1	0	0.363	1	0	**0.026***	1	0	**0.015***	1	0	0.073	0	1	**0.019***
mp	4	2		4	2		6	0		6	0		6	0		6	0		6	0		6	0	
se	38	8		23	23		32	14		43	3		44	2		44	2		45	1		42	4	
Si	7	2		5	4		6	3		7	2		6	3		6	3		7	2		7	2	
Stage																								
I	3	3	0.153	4	2	0.248	6	0	0.373	6	0	0.192	6	0	0.165	6	0	**0.015***	6	0	**0.013***	5	1	0.545
II	10	3		8	5		10	3		12	1		13	0		13	0		13	0		13	0	
III	26	3		11	18		19	10		28	1		27	2		28	1		29	0		25	4	
IV	11	3		9	5		10	4		11	3		11	3		10	4		11	3		12	2	

[a]See Table 1 for details.
*$P < 0.05$ (in boldface).

TABLE 4. Chromosomal Abnormalities in Gastric Carcinomas

Chromosomal abnormality[a]	Frequency of regional gain or loss (%)	Association with clinicopathologic features[b]	Candidate oncogenes/TSGs[c]
+1p32–p36	42	+	*JUN, BLYM1, EPS15, GAC1, MPL, MYCL1, LCK, PTP4A2, DVL1, LAP18, CDC42,*
+3q28-qter	8	+	*ETV5*
+6p21-pter	19	+	*CCND3, CBFA1, PPARD, PIM1, MAPK14, PBX2, NOTCH4, RFP, DEK, IRF4*
+7p21-pter	18	+	*IL6, GNA12, ETV1, PDGFA*
+8q23–q24	18	−	*FAK, PVT1, WISP1, NOV, PDNP2,* ***MYC***
+9q34	31	−	*VAV2, SET, RALGDS, ABL1, NUP214, GFIIB, NOTCH*
+11q12–q13	35	−	*PAK1, FGF3, SEA, CCND1, MYEOV, RELA, EMS1, FGF4*
+13q13–q14	8	+	*FKHR, LCP1*
+16p13-pter	21	+	*MRP, SSTR5*
+17p13-pter	24	+	*STK12/AIK2, ABR, CRK*
+17q12–q21	57	−	*WNT3, ETV4, TOB1, GRN*
+17q24-qter	53	+	*RAC3, GRB2, SSTR2, MAFG,* ***SURVIVIN***
+20p12-pter	19	+	*CDC25B,* ***PCNA***
+20q13-qter	48	+	*CAS, DCR3, SNAI1, MYBL2, GNAS1, NABC1, STK15/BTAK, ZNF217, CYP24, BRK*
+22q12-qter	27	+	*YWHAH, PDGFB, RAC2, TOB2*
−3p12	8	+	
−3p25-pter	8	+	*RAD18, TIMP4, ST11, RAD23B, XPC, VHL*
−4p14–p15	10	−	
−4q13–q32	11	−	*MAD2L1,MADH1/SMAD1*
−5q14–q23	8	+	***MSH3****, XRCC4, DRM, RAD17, RAS1/GAP, RASA1/GAP,* ***CTNNA1, APC****, RAD50,* ***MCC***
−9p21–p23	5	+	*INFs,* ***CDKN2A****, CDKN2B, P14ARF*
−13q21–q22	16	−	*BRCA4*
−18q12-qter	11	+	*EB2, OSTS, MADH2/SMAD2,* ***DCC****, MASPIN,* ***MADH4/SMAD4***

[a]Novel sites in GC are underlined.
[b]The relationship between DNA copy number changes to clinicopathologic features is shown in Table 3. "+" indicates $P < 0.05$, "−" indicates $P > 0.05$.
[c]The list of oncogenes/tumor-suppressor genes is based on the information provided on http://caroll.vjf.inserm.fr. Genes in boldface represent those that have been shown to be abnormal in GC.

gains on 13q and 20q manifested a mixed type of expanding and infiltrating growth pattern (P = 0.032 and 0.012). Losses from 3p25-pter, 5q, and 18q were associated with advanced stages of tumor invasion (T3 + T4) (P = 0.026, 0.015, and 0.019). In addition, gains on 1p and losses on 5q and 9p were associated with GC of advanced TNM stages (P = 0.023, 0.015, and 0.013). Metastatic GC was also shown to be associated with specific genetic alterations. Gain on 1p was associated with lymph node metastasis (P = 0.038), whereas losses from 3p25-pter and 5q were associated with liver metastasis (P = 0.030 and 0.030). Gain on 17p was associated with vascular invasion (P = 0.041), but gains on 6p and 20p were more frequently found in GC where lymphatic duct invasion was absent (P = 0.046 and 0.046). Loss from 3p12 was associated with peritoneal carcinoma (P = 0.026). When patient age was considered, we found that gain on 6p was predominantly associated with older patients (≥65 years) (P = 0.046), whereas gain on 3q was associated with relatively younger patients (<65 years) (P = 0.041). Gain on 16p occurred more frequently in tumors located at the upper third of the stomach (P = 0.021). Loss of 18q was associated with GC of lower nuclear grade (P = 0.007). A tendency of poorer prognosis was observed with the loss of 3p25-pter (P = 0.083). Multivariate analysis revealed that gain on 20q is an independent factor associated with the intestinal type of GC by Lauren's classification (P = 0.038). Gain on 1p is tightly associated with infiltrating-type GC (P = 0.005), whereas gain on 6p and loss of 18q were associated with expanding-type GC by Ming's criteria (P = 0.033 and 0.038). Gains on 7p, 13q, and 20p were associated with tumors of a specific infiltrating growth pattern (P = 0.018, 0.045 and 0.035). Loss from 3p25-pter and gain on 1p were tightly associated with advanced stages of tumor invasion (T3 + T4) (P = 0.018) and ad-

vanced TNM stages ($P = 0.026$), respectively. Loss from 3p25 was the most significant genetic change associated with liver metastasis ($P = 0.012$), whereas gain on 6p was an independent factor associated with tumors that lack lymphatic duct invasion ($P = 0.034$).

DISCUSSION

The present study represents the first detailed genome-wide investigation on genetic imbalances in a large panel of GCs in relation to clinicopathologic features. Chromosomal abnormalities were first identified in six gastric cancer cell lines, and the findings of gains in 1p, 11q, 17q, and 20q, and losses from 5q and 18q are in agreement with previous cytogenetic studies (Okada et al., 2000). These abnormalities are also consistently found in the primary GC specimens. Overall, we found 15 regions showing recurrent gains and 8 regions of frequent losses in primary tumors, with several sites being novel in GC. Statistical analyses revealed that gains at 17q24-qter (53%), 20q13-qter (48%), 1p32–p36 (42%), 22q12-qter (27%), 17p13-pter (24%), 16p13-pter (21%), 6p21-pter (19%), 20p12-pter (19%), 7p21-pter (18%), 3q28-qter (8%), and 13q13–q14 (8%), and losses at 18q12-qter (11%), 3p12 (8%), 3p25-pter (8%), 5q14–q23 (8%), and 9p21–p23 (5%) are associated with specific subtypes of GC with unique clinicopathologic features. These regions may contain critical genes involved in the pathogenic development of GC and are strong indicators of critical regions that warrant detailed investigation.

Amplification of 17q is the most frequent change found in this study, involving 61% of the cases tested, with two regions frequently amplified: 17q12–q21 and 17q24-qter. Chromosomal gain in 17q12–q21 has been described in many human cancers, including gastric cancer (Knuutila et al., 1998b). Concomitant amplification of the candidate genes *GAS* and *ERBB2* located at the 17q12–q21 region was frequently detected in the intestinal type of GC (Vidgren et al., 1999). Overexpression of *ERRB2* was shown to be associated with highly aggressive well-differentiated adenocarcinomas with an adverse prognosis (Sanz Ortega et al., 2000). As for the distal region of the long arm of chromosome 17, it is amplified in nasopharyngeal carcinoma (Chen et al., 1999), neuroblastoma (Bown et al., 1999), and malignant peripheral nerve sheath tumors (MPNSTs) (Schmidt et al., 1999). Gain of 17q21-qter predicts advanced disease and a poor outcome in neuroblastoma (Bown et al., 1999), and high-level amplification of 17q24-qter predicts poor overall survival in MPNST (Schmidt et al., 1999, 2000). Consistent with these reports, amplification of 17q24-qter found in 53% of GCs was associated with the infiltrative subtype of GC by Ming's criteria, a poor prognostic factor predicting adverse outcome, suggesting that the region of 17q24-qter may harbor dominant candidate genes governing the mode of tumor growth and invasion. Candidate genes located in this region include genes encoding the small GTP binding protein RAC3, growth factor receptor binding protein 2 (GRB2), somatostatin receptor 2, protein G basic leucine zipper transcription MafG, and Survivin. Survivin encodes a member of the inhibitor of apoptosis proteins family, and high expression was shown to promote cell survival in neuroblastomas and was significantly associated with a poor prognosis (Islam et al., 2000).

In this study, we also confirmed previous reports of chromosomal gains on 20q, 8q, and 20p, and losses from 4q, 18q, and 5q as frequent events in GC (Kokkola et al., 1997; El-Rifai et al., 1998; Sakakura et al., 1999; van Dekken et al., 1999). We further showed that gains of 20q and 20p and losses of 18q and 5q were strongly associated with unique features of tumor- or patient-related factors, demonstrating the important roles of these genetic events in the initiation and/or progression of specific subtypes of GC. Amplification of 20q13-qter has been reported in a broad range of tumors, including cancers of the breast, esophagus, nasopharynx, kidney, prostate, colorectum, pancreas, and salivary gland (Knuutila et al., 1998b). Amplification of 20q was associated with GC of the intestinal type by Lauren's classification (Kokkola et al., 1997). In hepatocellular carcinoma, 20q amplification was associated with larger tumor size (Guan et al., 2000). Gain in 20q was suggested to promote cell immortalization (Cuthill et al., 1999).

This region has been suggested to contain one or more genes that are overexpressed in epithelial cancers. In breast cancer, serine/threonine kinase *STK15*, nonreceptor protein tyrosine kinase *BRK*, and vitamin D hydroxylase *CYP24* are frequently amplified and overexpressed (Anzick et al., 1997; Pinkel et al., 1998; Albertson et al., 2000). The human cellular apoptosis susceptibility gene *CAS* has also been mapped to this region (Brinkmann, 1998). Amplification of 20p12-pter was shown to be associated with the expanding subtype of GC, and candidate genes in this region include *CDC25B* and *PCNA*, each of which plays an important role at specific stages of cell cycle progression. Deletions of 5q and 18q have been reported in many human cancers (Knuutila et al., 1998b). Inactivation of *APC*, located at 5q21, has been shown to initiate the majority of colorectal cancers, including famil-

ial adenomatosis polyposis (Miyoshi et al., 1992; Powell et al., 1993). In contrast, allelic deletion of *APC/MCC* has been shown to be a frequent but late event in human gastric carcinogenesis (Rhyu et al., 1994). Our finding that loss of 5q14–q23 was associated with tumors with serosa invasion (T3 + T4) and higher TNM staging confirmed *APC* loss to be a late event in gastric tumor progression. The close association between 5q loss and liver metastasis further illustrates the importance of this event in the process of tumor invasion. LOH on 18q predicted poor survival in colorectal cancer, pancreatic cancer, and head and neck squamous cell carcinoma (Ogunbiyi et al., 1998; Pearlstein et al., 1998; Yatsuoka et al., 2000), and this study confirms previous reports that LOH of 18q is associated with tumor progression and poor prognosis of GC (Inoue et al., 1998).

We have also identified other genetic abnormalities previously unreported in GC. Among them, recurrent gains of 1p32–p36, 6p21-pter, 13q13–q14, 16p13-pter, and losses of 3p25-pter were found to be independent genetic changes strongly associated with subsets of GC with unique clinicopathological features, suggesting the presence of additional genes involved in the pathogenesis of GC. By multivariate analysis, we found that gain in 1p32–p36 was associated with tumors of lymph node metastasis and higher TNM stages, suggesting it to be a late event in GC progression. In addition, it was also associated with GC of the infiltrative subtype by Ming's criteria, suggesting that overexpression of genes located in this region may contribute to aggressive tumor growth and invasiveness. Amplification of 6p21-pter was an independent factor associated with the expanding type of GC by Ming's classification, and it was also associated with tumors that lacked lymphatic duct invasion. As for 13q, deletion of the *RB1* locus at 13q14 was not obvious by CGH analysis. Instead, amplification of this locus was observed, suggesting the presence of dominant oncogenes in this region. Amplification of 16p13-pter was the only genetic abnormality shown to be associated with tumor site, that is, the gastric cardia. The loss of 3p25-pter was associated with the depth of tumor invasion and liver metastasis, making it a late event in GC progression. In agreement with our previous report that depth of tumor invasion is a poor prognostic factor (Wu et al., 1996b), GC with loss of 3p25-pter showed a tendency toward poor survival, although this did not reach statistical significance.

The initiation and progression of GC involve multiple genetic alterations. Although many chromosomal alterations have been reported in association with GC development and progression, genes localized to those altered regions relating to GC pathogenesis have not yet been identified. Other than the commonly detected regions, we have also identified regions that have not been identified in previous reports. Specific chromosomal abnormalities were found to be strongly associated with subtypes of GC portraying unique clinicopathologic features. Our findings warrant further studies to identify potential candidate genes involved in the pathogenic development of GC, and the regions of clinicopathologic association represent regions most likely to contain genes of biological significance for GC development and progression.

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REFERENCES

Albertson DG, Ylstra B, Segraves R, Collins C, Dairkee SH, Kowbel D, Kuo W-L, Gray JW, Pinkel D. 2000. Quantitative mapping of amplicon structure by array CGH identifies CYP24 as a candidate oncogene. Nat Genet 25:144–146.

Anzick SL, Kononen J, Walker RL, Azorsa DO, Tanner MM, Guan XY, Sauter G, Kallioneimi O-P, Trent JM, Meltzer PS. 1997. AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. Science 277:965–968.

Bown N, Cotterill S, Lastowska M, O'Neill S, Pearson AD, Plantaz D, Meddeb M, Danglot G, Brinkschmidt C, Christiansen H, Laureys G, Speleman F. 1999. Gain of chromosome arm 17q and adverse outcome in patients with neuroblastoma. N Engl J Med 340:1954–1961.

Brinkmann U. 1998. CAS, the human homologue of the yeast chromosome-segregation gene CSE1, in proliferation, apoptosis, and cancer. Am J Hum Genet 62:509–513.

Brito MJ, Filipe MI, Williams GT, Thompson H, Ormerod MG, Titley J. 1993. DNA ploidy in early gastric carcinoma (T1): a flow cytometric study of 100 European cases. Gut 34:230–234.

Chen Y-J, Ko J-Y, Chen P-J, Shu C-H, Hsu M-T, Tsai S-F, Lin C-H. 1999. Chromosomal aberrations in nasopharyngeal carcinoma analyzed by comparative genomic hybridization. Genes Chromosomes Cancer 25:169–175.

Cuthill S, Agarwal P, Sarkar S, Savelieva E, Reznikoff CA. 1999. Dominant genetic alterations in immortalization: role for 20q gain. Genes Chromosomes Cancer 26:304–311.

Dos Santos NR, Seruca R, Constancia M, Seixas M, Sobrinho-Simoes M. 1996. Microsatellite instability at multiple loci in gastric carcinoma: clinicopathologic implications and prognosis. Gastroenterology 110:38–44.

El-Rifai W, Harper JC, Cummings OW, Hyytinen E-R, Frierson HF, Knuutila S, Powell SM. 1998. Consistent genetic alterations in xenografts of proximal stomach and gastro-esophageal junction adenocarcinomas. Cancer Res 58:34–37.

Guan XY, Fang Y, Sham JS, Kwong DL, Zhang Y, Liang Q, Li H, Zhou H, Trent JM. 2000. Recurrent chromosome alterations in hepatocellular carcinoma detected by comparative genomic hybridization. Genes Chromosomes Cancer 29:110–116.

Han K, Oh EJ, Kim YS, Kim YG, Lee KY, Kang CS, Kim BK, Kim WI, Shim SI, Kim SM. 1996. Chromosomal numerical aberrations in gastric carcinoma: analysis of eighteen cases using in situ hybridization. Cancer Genet Cytogenet 92:122–129.

Inoue T, Uchino S, Shiraishi N, Adachi Y, Kitano S. 1998. Loss of heterozygosity on chromosome 18q in cohesive-type gastric cancer is associated with tumor progression and poor prognosis. Clin Cancer Res 4:973–977.

Islam A, Kageyama H, Takada N, Kawamato T, Takayasu H, Isogai E, Ohira M, Hashizume K, Kobayashi H, Kaneko Y, Nakagawara A. 2000. High expression of Survivin, mapped to 17q25, is significantly associated with poor prognostic factors and promotes cell survival in human neuroblastoma. Oncogene 19:617–623.

Kallioniemi O-P, Kallioniemi A, Piper J, Isola J, Waldman FM, Gray JW, Pinkel D. 1994. Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumor. Genes Chromosomes Cancer 10:231–243.

Kihana T, Tsuda H, Hirota T, Shimosato Y, Sakamoto H, Terada M, Hirohashi S. 1991. Point mutation of c-Ki-ras oncogene in gastric adenoma and adenocarcinoma with tubular differentiation. Jpn J Cancer Res 82:308–314.

Kim J-H, Takahashi T, Chiba I, Park J-G, Birrer MJ, Roh JK, Lee HD, Kim J-P, Minna JD, Gazdar AF. 1991. Occurrence of p53 gene abnormalities in gastric carcinoma tumors and cell lines. J Natl Cancer Inst 83:938–943.

Knuutila S, Armengol G, Bjorkqvist A-M, El-Rifai W, Larramendy ML, Monni O, Szymanska J. 1998a. Comparative genomic hybridization study on pooled DNAs from tumors of one clinical-pathological entity. Cancer Genet Cytogenet 100:25–30.

Knuutila S, Bjorkqvist A-M, Autio K, Tarkkanen M, Wolf M, Monni O, Szymanska J, Larramendy ML, Tapper J, Pere H, El-Rifai W, Hemmer S, Wasenius V-M, Vidgren V, Zhu Y. 1998b. DNA copy number amplifications in human: review of comparative genomic hybridization studies. Neoplasms. Am J Pathol 152:1107–1123.

Kobayashi M, Kawashima A, Mai M, Ooi A. 1996. Analysis of chromosome 17p13 (p53 locus) alterations in gastric carcinoma cells by dual-color fluorescence in situ hybridization. Am J Pathol 149:1575–1584.

Kokkola A, Monni O, Puolakkainen P, Larramendy ML, Victorzon M, Nordling S, Haapiainen R, Kivilaakso E, Knuutila S. 1997. 17q12–21 amplicon, a novel recurrent genetic change in intestinal type of gastric carcinoma: a comparative genomic hybridization study. Genes Chromosomes Cancer 20:38–43.

Labrecque S, Naor N, Thomson D, Matlashewski G. 1993. Analysis of the anti-p53 antibody response in cancer patients. Cancer Res 53:3468–3471.

Lauren P. 1965. The two histological main types of gastric carcinoma. Diffuse and so-called intestinal type of carcinoma: an attempt at a histoclinical classification. Acta Pathol Microbiol Scand 64:31–49.

Lin J-T, Wu M-S, Shun C-T, Lee W-J, Wang J-T, Wang T-H, Sheu J-C. 1995. Microsatellite instability in gastric carcinoma with special references to histopathology and cancer stages. Eur J Cancer 31:1879–1882.

Ming S-C. 1977. Gastric carcinoma: a pathobiological classification. Cancer 39:2475–2485.

Miyoshi Y, Nagase H, Ando H, Horii A, Ichii S, Nakatsuru S, Aoki T, Miki Y, Mori T, Nakamura Y. 1992. Somatic mutations of the APC gene in colorectal tumors: mutation cluster region in the APC gene. Hum Mol Genet 1:229–233.

Mizutani T, Onda M, Tokunaga A, Yamanaka N, Sugisaki Y. 1993. Relationship of c-erbB-2 protein expression and gene amplification to invasion and metastasis in human gastric cancer. Cancer 72:2083–2088.

Nakatani H, Sakamoto H, Yoshita T, Yokota J, Tahara E, Sugimura T, Terada M. 1990. Isolation of an amplified DNA sequence in stomach cancer. Jpn J Cancer Res 81:707–710.

Ogunbiyi OA, Goodfellow PJ, Herfarth K, Gagliardi G, Swanson PE, Birnbaum EH, Read TE, Fleshman JW, Kodner IJ, Moley JF. 1998. Confirmation that chromosome 18q allelic loss in colon cancer is a prognostic indicator. J Clin Oncol 16:427–433.

Okada K, Sugihara H, Bamba M, Bamba T, Hattori T. 2000. Sequential numerical changes of chromosomes 7 and 18 in diffuse-type stomach cancer cell lines: combined comparative genomic hybridization, fluorescence in situ hybridization, and ploidy analyses. Cancer Genet Cytogenet 118:99–107.

Pearlstein RP, Benninger MS, Carey TE, Zarbo RJ, Torres FX, Rybicki BA, Dyke DL. 1998. Loss of 18q predicts poor survival of patients with squamous cell carcinoma of the head and neck. Genes Chromosomes Cancer 21:333–339.

Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo W-L, Chen C, Zhai Y, Dairkee SD, Ljung BM, Gray JW, Albertson DG. 1998. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nat Genet 20:207–211.

Powell SM, Petersen GM, Krush AJ, Booker S, Jen J, Giardiello FM, Hamilton SR, Vogelstein B, Kinzler KW. 1993. Molecular diagnosis of familial adenomatous polyposis. N Engl J Med 329:1982–1987.

Renault B, van der Broek M, Fodde R, Wijnen J, Pellegata NS, Amadori D, Khan PM, Ranzani GN. 1993. Base transitions are the most frequent genetic changes at p53 in gastric cancer. Cancer Res 53:2614–2617.

Rhyu MG, Park WS, Jung YJ, Choi SW, Meltzer SJ. 1994. Allelic deletions of MCC/APC and p53 are frequent late events in human gastric carcinogenesis. Gastroenterology 106:1584–1588.

Rooney PH, Murray GI, Stevenson DA, Haites NE, Cassidy J, McLeod HL. 1999. Comparative genomic hybridization and chromosomal instability in solid tumors. Br J Cancer 80:862–873.

Sakakura C, Mori T, Sakabe T, Ariyama Y, Shinomiya T, Date K, Hagiwara A, Yamaguchi T, Takahashi T, Nakamura Y, Abe T, Inazawa J. 1999. Gains, losses, and amplifications of genomic materials in primary gastric cancers analyzed by comparative genomic hybridization. Genes Chromosomes Cancer 24:299–305.

Sambrook J, Fritsch E, Maniatis T. 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Sanz Ortega J, Steinberg SM, Moro E, Saez M, Lopez JA, Sierra E, Sanz Esponera J, Merino MJ. 2000. Comparative study of tumor angiogenesis and immunohistochemistry for p53, c-ErbB2, c-myc and EGFR as prognostic factors in gastric cancer. Histol Histopathol 15:455–462.

Schmidt H, Wurl P, Taubert H, Meye A, Bache M, Holzhausen HJ, Hinze R. 1999. Genomic imbalances of 7p and 17q in malignant peripheral nerve sheath tumors are clinically relevant. Genes Chromosomes Cancer 25:205–211.

Schmidt H, Taubert H, Meye A, Wurl P, Bache M, Bartel F, Holzhausen HJ, Hinze R. 2000. Gains in chromosomes 7, 8q, 15q, and 17q are characteristic changes in malignant but not in benign peripheral nerve sheath tumors from patients with Recklinghausen's disease. Cancer Lett 155:181–190.

Soman NR, Correa P, Ruiz BA, Wogan GN. 1991. The TPR-MET oncogenic rearrangement is present and expressed in human gastric carcinoma and precursor lesions. Proc Natl Acad Sci USA 88:4892–4896.

Tahara E. 1995. Molecular biology of gastric cancer. World J Surg 19:484–490.

Tamura G, Maesawa C, Suzuki Y, Ogasawara S, Terashima M, Saito K, Satodate R. 1993. Primary gastric carcinoma cells frequently lose heterozygosity at the APC and MCC genetic loci. Jpn J Cancer Res 84:1015–1018.

Uchino S, Tsuda H, Maruyama K, Kinoshita T, Sasako M, Saito T, Kobayashi M, Hirohashi S. 1993. Overexpression of c-erbB-2 protein in gastric cancer. Its correlation with long-term survival of patients. Cancer 72:3179–3184.

van Dekken H, Geelen E, Dinjens WN, Wijnhoven BP, Tilanus HW, Tanke HJ, Rosenberg C. 1999. Comparative genomic hybridization of cancer of the gastroesophageal junction: deletion of 14q31–32.1 discriminates between esophageal (Barrett's) and gastric cardia adenocarcinomas. Cancer Res 59:748–752.

Vidgren V, Varis A, Kokkola A, Monni O, Puolakkainen P, Nordling S, Forozan F, Kallioniemi A, Vakkari M-L, Kivilaskso E, Knuutila S. 1999. Concomitant gastrin and ERBB2 gene amplifications at 17q12–q21 in the intestinal type of gastric cancer. Genes Chromosomes Cancer 24:24–29.

Whelan SL, Parkin DM, Masuyer E. 1993. Trends in cancer incidence and mortality. Lyon, France: IARC Scientific.

Wu C-W, Hsieh M-C, Lo S-S, Lui W-Y, P'eng F-K. 1996a. Results of curative gastrectomy for carcinoma of the distal third of the stomach. J Am Coll Surg 183:201–207.

Wu C-W, Hsieh M-C, Lo S-S, Tsay S-H, Lui W-Y, P'eng F-K. 1996b. Relation of number of positive lymph nodes to the prognosis of patients with primary gastric adenocarcinoma. Gut 38:525–527.

Wu C-W, Hsieh M-C, Lo S-S, Tsay S-H, Li A-F, Lui W-Y, P'eng F-K. 1997. Prognostic indicators for survival after curative resection for patients with carcinoma of the stomach. Dig Dis Sci 42:1265–1269.

Wu M-S, Lee C-W, Shun C-T, Wang H-P, Lee W-J, Sheu J-C, Lin J-T. 1998. Clinicopathological significance of altered loci of replication error and microsatellite instability-associated mutations in gastric cancer. Cancer Res 58:1494–1497.

Wu M-S, Lee C-W, Shun C-T, Wang H-P, Lee W-J, Chang M-C, Sheu J-C, Lin J-T. 2000. Distinct clinicopathologic and genetic profiles in sporadic gastric cancer with different mutator phenotypes. Genes Chromosomes Cancer 27:403–411.

Yatsuoka T, Sunamura M, Furukawa T, Fukushige S, Yokoyama T, Inoue H, Shibuya K, Takeda K, Matsuno S, Horii A. 2000. Association of poor prognosis with loss of 12q, 17p, and 18q, and concordant loss of 6q/7p and 12q/18q in human pancreatic ductal adenocarcinoma. Am J Gastroenterol 95:2080–2085.